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Detection of *Rickettsia aeschlimannii* in *Hyalomma marginatum* ticks in western Russia

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INTRODUCTION

SFG rickettsiae are obligate intracellular Gram-negative bacteria. These bacteria are associated with arthropods, mainly ticks, and can be transmitted to mammals and humans. Ixodid ticks of the *Hyalomma marginatum* species are widely spread in semi-desert and steppe landscape areas of south-western Russia, in particular in the Stavropol region where they have been reported to bite humans [1]. The main hosts of the larvae and nymphs of this tick species include several species of wild and domestic birds, the European brown hare, and four-toed and eared hedgehogs. In contrast, adult ticks parasitise livestock and domestic dogs, and may attack humans. In Russia, *H. marginatum* ticks are known to be the main vectors of the Crimean-Congo haemorrhagic fever (CCHF) virus in the southern part of the country. In addition, strains of West Nile fever virus were isolated from *H. marginatum* ticks collected in the Volga delta in Astrakhan. However, to date, the presence of rickettsiae in *H. marginatum* has not been evaluated. In the present study, we investigated by PCR the presence of rickettsiae in *H. marginatum* ticks collected in the Stavropol region of Russia.

MATERIALS AND METHODS

Forty adult *H. marginatum* ticks (i.e. 20 females and 20 males) were collected from cattle and on the vegetation in the semi-desert areas of the Stavropol region of Russia in May 2004. DNA was extracted individually from each tick using the QIAmp Tissue Kit as proposed by the manufacturer (Qiagen, Hilden, Germany). PCR-amplification of the complete *gltA* gene was attempted using the CSf (TAT GAC CAA TGA AAA TAA TAA) and CSFin (ACT TAT ACT CTC TAT GTA C) primers, and a 590-bp fragment of the *ompA* gene, specific for spotted fever group rickettsiae, was amplified using the Rr190.70p and Rr190.701n primers as previously described [2]. PCR were carried out in a Peltier 20 model PTC-200 thermal cycler (MJ Research, Inc., Watertown, MA, USA). Positive PCR products were purified using the QIAquick PCR purification kit (Qiagen) as recommended by the manufacturer and sequenced using PCR primers and the d-Rhodamine Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Warrington, UK) and an ABI 3100 PRISM automated sequencer (Applied Biosystems). Sequences were identified by comparison with GenBank entries.

RESULTS

Eight out of 40 tested *H. marginatum* ticks (20%) (i.e. five females and three males) were PCR-positive for both genes. Negative controls remained negative. PCR products had the expected sizes. For each gene, all eight PCR products exhibited identical nucleotide sequences. *gltA* and *ompA* nucleotide sequences obtained from all eight ticks were 99.9% and 99.8% similar, respectively, to those of *R. aeschlimannii* strain MS16T (GenBank accession numbers U59722 and U43800). Thus, using the genetic criteria previously developed to determine the taxonomic status of rickettsial isolates [3], we could classify these tick amplicons within the *R. aeschlimannii* species. Sequences obtained in our study were deposited in GenBank under numbers DQ235776 and DQ235777, for the *gltA* and *ompA* genes, respectively.

DISCUSSION

To the best of our knowledge, this is the first detection of *R. aeschlimannii* in ticks in Russia. To date, *R. aeschlimannii* has been detected in

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Fig. 1. World map showing the distribution of *R. aeschlimannii* (red dots).

H. marginatum ticks collected in Africa (Mali, Niger, Morocco, Tunisia, Zimbabwe) and Europe (Croatia, France, Greece, Portugal, Spain) (Fig. 1) [4]. In this tick species, rickettsiae were shown to be transtadially and transovarially transmitted. In addition, *R. aeschlimannii* has been found in *Hyalomma dromedarii* and *Hyalomma truncatum* ticks collected in Sudan, and a rickettsia closely related to *R. aeschlimannii* has been found in *Haemaphysalis punctata* ticks in Kazakhstan. In 2002, the pathogenic role of *R. aeschlimannii* was demonstrated in a traveller who returned from Morocco with a febrile maculo-papular rash and a single eschar [5], and then in a hunter in South Africa who only developed an inoculation eschar. However, it was recently demonstrated that the risk of disease following bites of *R. aeschlimannii*-infected *H. marginatum* was low [4].

Our study expands the knowledge on pathogenic tick-borne rickettsiae in Russia, including *R. sibirica* subsp. *sibirica*, causing North Asian tick typhus, *R. conorii* subsp. *caspia*, responsible for Astrakhan fever, *R. heilongjiangensis*, agent of far-eastern rickettsiosis, and *R. slovaca* and *R. raoultii*, causing tick-borne lymphangitis, and *R. aeschlimannii* [4]. This list may also include the putative pathogen *R. helvetica*.

REFERENCES

1. Kotti BK, Shaposhnikova LI, Evchenko IM *et al*. *Hyalomma marginatum* Koch in the Stavropol region. *Zh Mikrobiol Epidemiol Immunobiol* 2001; **6:** 105–108.
2. Fournier PE, Roux V, Raoult D. Phylogenetic analysis of spotted fever group rickettsiae by study of the outer surface protein rOmpA. *Int J Syst Bacteriol* 1998; **48:** 839–849.
3. Fournier PE, Dumler JS, Greub G, Zhang J, Yimin W, Raoult D. Gene sequence-based criteria for the identification of new *Rickettsia* isolates and description of *Rickettsia heilongjiangensis* sp. nov. *J Clin Microbiol* 2003; **41:** 5456–5465.
4. Parola P, Paddock CD, Raoult D. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. *Clin Microbiol Rev* 2005; **18:** 719–756.
5. Raoult D, Fournier PE, Abboud P, Caron F. First documented human *Rickettsia aeschlimannii* infection. *Emerg Infect Dis* 2002; **8:** 748–749.